Nickel-induced Alterations in Human Renal Epithelial Cells

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Cellular progression to malignancy appears to require a number of distinct steps in which genetic damage in key regulatory genes accumulates. Immortalization, or escape from senescence, is considered to be one of the first phenotypic changes. Ni^{2+} treatment of normal human kidney epithelial (NHKE) cells *in vitro* resulted in immortalization of the cells (IHKE cells). The combined action of Ni^{2+} and v-Ha-*ras* oncogene fully transformed the cells to tumorigenicity in athymic nude mice. Sequence analysis of DNA from IHKE cells revealed point mutation in the *p53* gene at codon 238 with T→C transition. These findings suggest that Ni-induced mutation in the *p53* gene can be involved in the immortalization of the NHKE cells. The results also show that changes in the responses to EGF and TGFβ and in the expression of their receptors occur during malignant progression *in vitro*. — Environ Health Perspect 102(Suppl 3):117–118 (1994).

Key words: nickel carcinogenesis, immortalization, malignant transformation, karyotype, *p53*, EGF, TGFβ

Introduction

Substantial amounts of potentially toxic metals have been introduced into the environment. There is a concern that workers exposed to some metals might have an increased incidence of cancer. A number of epidemiologic and laboratory animal studies have demonstrated the carcinogenicity of nickel compounds (*1–3*). However, little is known about the molecular mechanism(s) of nickel carcinogenesis. Tumor development consists of multistep accumulations of both genetic and epigenetic changes. Accumulating evidence indicates that mutations in the *p53* gene are the most common genetic change in human cancers (*4*). Recent studies indicate that nickel carcinogenesis may be mediated through oxidative genotoxic effects (*5,6*). Higinbotham et al. found that nickel subsulfide induces GGT to GTT transversions in codon 12 of the K-*ras* oncogene in rat renal sarcomas (*7*).

In vitro model systems have been developed to facilitate the study of carcinogenesis, i.e., the causative agents of neoplasia and cancer progression at the cellular and molecular genetic levels. We have recently developed an *in vitro* human multistep model for the study of human epithelial carcinogenesis (*8,9*). The present report describes results obtained following nickel treatment of normal diploid human kidney epithelial cells and subsequent transfection with the v-Ha-*ras* oncogene.

Materials and Methods

Cells and Cell Culture

Normal human kidney epithelial (NHKE) cells were initiated from kidney explants of fetuses from midtrimester therapeutic abortions (*8*). Cells were routinely cultured in a MEM medium supplemented with EGF (10 ng/ml), insulin (5 µg/ml), hydrocortizone (5×10^{-7}M), transferrin (5 µg/ml), and 5% FCS. The immortalized human kidney epithelial cell line ($_1$IHKE) and tumorigenic human kidney epithelial ($_2$THKE) cell lines were cultured with the same medium, but FCS was reduced to 1% (*8,9*).

Transformation Protocol

NHKE cells were transformed into immortal cell line (IHKE) by continous exposure to Ni^{2+} (5 µg/ml) for 70 to 100 days (*8,9*). The IHKE cells were transfected with pZip-*ras* DNA and positive clones were selected by G418 sulfate (*9*).

Tumorigenicity Evaluation

IHKE cells and G418-resistant cell clones were tested for tumorigenicity in athymic nude mice.

p53 Analysis

Location of p53 protein was assayed immunocytochemically with monoclonal antibodies Pab 1801 and Pab 421 (Oncogene Science, Inc., Manchester, NY). Details of the PCR amplification and sequencing have been published elsewhere (*10*).

Results and Discussion

The immortalization of NHKE cells by Ni^{2+} has been described in detail by Tveito et al. (*8*). Following Ni^{2+} treatment, the cells aquired an indefinite life-span in culture, produced colonies in soft agar, but did not undergo neoplastic transformation.

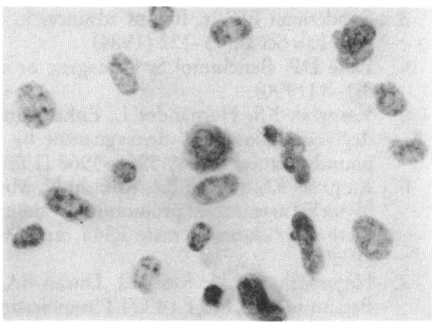

Figure 1. Immunohistochemical staining of p53 in IHKE cells x630. Reproduced with permission from Mæhle et al. (*10*).

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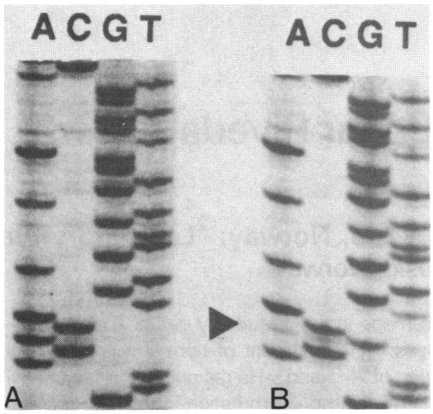

Figure 2. Genomic analysis of the *p53* gene (A) wild-Otype sequence; (B) IHKE cells with a T→C transition in codon 238. Reproduced with permission from Mæhle et al. (*10*).

Common for the IHKE cells were abnormalities of chromosomes 1, 7, 9, 11, 13, 14, and 20, increased number of chromosome 17, and loss of normal chromosomes 20 and 22 (*8*).

The studies were extended to include transfection of the IHKE cells with the plasmid pZipNeo SV(X) containing the v-Ha-*ras* gene. We found that cells derived from the G418-resistant cell clones formed tumors in athymic mice. The cells from Ni-treated cells produced no tumors in this assay (*9*).

At present little is known about the progression of cells from normal proliferative control to malignancy. There is, however, increasing evidence linking development and progression of cancer to accumulation of mutations (*11,12*). Alterations of functional genes associated with cellular proliferation and differentiation, i.e., oncogenes and tumor suppressor genes, are required during oncogenesis leading to deregulation of the mitogenic program.

One gene implicated in the pathogenesis of human cancer is the *p53* gene. Studies suggest that the *p53* gene is a molecular target for genetic damages caused by environmental factors (*13*). The *p53* gene is located on band 13p of chromosome 17 and acts as a tumor suppressor. In an attempt to explore targets of mutation by nickel, we examined whether mutation in the *p53* gene has occurred in IHKE cells. Immunochemistry and sequence analysis of DNA from IHKE cells revealed abnormal p53 expression and a T→C transition mutation in codon 238. Our studies show that mutation of the *p53* gene are not sufficient for tumor formation, but the combined action of Ni^{2+} and transfection of the v-Ha-*ras* oncogene converted the immortalized cells to tumorigenic cells. Studies have shown that mutant *p53* gene in cooperation with Ha-*ras* gene transforms rodent fibroblasts to neoplastic cells (*14*).

Cell growth *in vitro* is controlled by growth factors. The IHKE and THKE cells demonstrated altered growth capacity in serum-free media. In recent years, it has become apparent that TGFβ and EGF play an important role in the regulation of cell growth of both normal and malignant epithelial cells. TGFβ consists of a family of closely related polypeptides with multiple biologic activities (*15*). It is a potent inhibitor of normal epithelial cell growth. TGFβ can also induce proliferation of some cell types, such as fibroblastic cells. Malignant epithelial cells may be resistant to the growth-inhibitory effects of TGFβ. Studies indicate that this resistance to TGFβ may be an important step in carcinogenesis (*15*). EGF influences many biologic responses of both normal and malignant cells and EGF-receptor overexpression has been shown in human malignant epithelial cell lines and tumors (*16*). In preliminary studies we have found that the IHKE cells are resistant to high concentrations of TGFβ. On the other hand, the normal cells were inhibited by TGFβ. NHKE cells were stimulated by EGF. In contrast, IHKE cells showed reduced sensitivity to growth stimulation by EGF. NHKE cells had fewer EGF receptors than IHKE cells and IHKE cells had fewer TGFβ receptors than NHKE cells (S. Mollerup et al., unpublished). We are currently examining in detail the importance of EGF and TGFβ in the progression to malignancy.

In conclusion, we have shown that Ni^{2+} induces immortalization and mutation in the *p53* tumor suppressor gene in normal human epithelial cells. Furthermore, preliminary data suggest that the Ni-induced transformation is associated with abrogation of EGF and TGFβ growth control.

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